

WHAT IS CLAIMED IS:

1. A method for isolation of biological macromolecules, said method comprising contacting at least one filter with a biological sample comprising the biological macromolecules of interest, wherein the pore size of said filter increases in the direction of sample flow.

2. The method of claim 1, wherein said biological sample is a cellular lysate.

3. The method of claim 2, wherein said cellular lysate is derived from eukaryotic cells.

4. The method of claim 2, wherein said cellular lysate is derived from prokaryotic cells.

5. The method of claim 3, wherein said eukaryotic cells are selected from the group consisting of fungi, fish cells, yeast cells, plant cells and animal cells.

6. The method of claim 1, wherein said biological macromolecules are nucleic acid molecules.

7. The method of claim 1, wherein said biological macromolecules are protein molecules.

8. The method of claim 6, wherein said nucleic acid molecules are RNA molecules.

9. The method of claim 8, wherein said RNA molecules are mRNA molecules.
10. The method of claim 6, wherein said nucleic acid molecules are DNA molecules.
11. The method of claim 10, wherein said DNA molecules are vectors or plasmids.
12. The method of claim 1, wherein said filter comprises at least two filter layers.
13. The method of claim 12, wherein a first filter has a pore size smaller than the second filter, and wherein said sample first contacts said first filter layer and then contacts said second filter layer.
14. The method of claim 13, wherein said second filter layer comprises at least one frit.
15. The method of claim 14, wherein said second filter layer comprises pores of sufficient size to shear genomic DNA, and said pore size is larger than that of the first filter layer.
16. The method of claim 15, wherein said pore size of said second filter layer is about 1 μm to 500 μm .
17. The method of claim 16, wherein said pore size of said second filter layer is about 10 μm to 70 μm .

18. The method of claim 17, wherein said pore size of said second filter layer is about 20 μm .

19. The method of claim 14, wherein said second filter layer comprises two frits.

20. The method of claim 19, wherein each of said frits are about 1/16 inch thick.

21. The method of claim 13, wherein said first filter layer comprises pores of sufficient size to retard the flow of cellular debris and particles.

22. The method of claim 21, wherein said pores of said first filter layer are about 0.1 μm to 1.0 μm in diameter.

23. The method of claim 21, wherein said pores of said first filter layer are about 0.2 μm in diameter.

24. The method of claim 13, wherein said second filter layer is comprised of polyethylene, polypropylene or a combination thereof.

25. The method of claim 13, wherein said first filter layer is comprised of one or more materials selected from the group consisting of hydrophobic polysulfone, hydrophilic polyether sulfone, cellulose, acetylated cellulose, nitrocellulose, polyester, polyolefin, scintered polyethylene, porous ceramics, silica, and polysaccharide.

26. The method of claim 25, wherein said first filter layer is comprised of regenerated cellulose.

27. The method of claim 26, wherein said first filter layer is comprised of regenerated cellulose, with a pore size of about 0.2 μm , and comprised of polyethylene or polypropylene, with an average pore size of about 20 μm .

28. The method of claim 1, wherein said filter is provided in a form selected from the group consisting of wafer, cylindrical, rectangular, beads, gels, square, cartridge, swab tip, plug, frit, membrane, sheets or inserts.

29. The method of claim 1, wherein said filter is provided in a form that is suitable to be inserted into a tube, microspin tube, microfuge tube, spin cartridge, vial, ampule, bag or suitable to fit multi-well plates typically used in processing of multiple samples, including, 6-well plates, 12-well plates, 24-well plates, 48-well plates, 96-well plates, 384-well plates, and the like, or suitable to fit into other plate sizes such as 35 mm plates, 60 mm plates, 100 mm plates, 150 mm plates, and the like.

30. The method of claim 1, wherein the flow of the sample is facilitated by centrifugation, gravity, pressure, vacuum, or any combination thereof.

31. A method for isolation of biological macromolecules, said method comprising;

(a) contacting cells or cellular source containing the macromolecules of interest with a composition capable of lysing all or substantially all of said cells to give a lysate; and

(b) contacting the lysate with a filter, wherein the filter comprises two or more filters, and wherein the pore size increases in the direction of sample flow; and

(c) promoting the flow of the sample through the filter.

32. An apparatus for use in isolating biological macromolecules, comprising one or more filters, wherein the pore size of said filters increases in the direction of sample flow.

33. The apparatus of claim 32, further comprising at least a first and second populations of pores.

34. The apparatus of claim 33, wherein said first population comprises pores of sufficient size to retard the flow of cellular debris, and said second population, which is downstream of said first population, comprises pores of sufficient size to shear genomic DNA.

35. The apparatus of claim 32, wherein a first and second filter are contained in a cartridge housing adapted to allow said first filter to be contacted with a sample comprising biological macromolecules before said second filter is contacted.

36. The apparatus of claim 35, wherein said second filter is a frit.

37. The apparatus of claim 36, wherein said second filter comprises pores of sufficient size to shear genomic DNA.

38. The apparatus of claim 37, wherein said pore size of said second filter is about 1 μm to 500 μm .

39. The apparatus of claim 38, wherein said pore size of said second filter is about 10 μm to 70 μm .

40. The apparatus of claim 39, wherein said pore size of said second filter is about 20 μm in diameter.

41. The apparatus of claim 37, wherein said first filter comprises pores of sufficient size to retard the flow of cellular debris and particles.

42. The apparatus of claim 41, wherein said pore size of said first filter is about 0.1 μm to 1.0 μm .

43. The apparatus of claim 42, wherein said pore size of said first filter is about 0.2 μm .

44. The apparatus of claim 36, wherein said frit is comprised of polyethylene, polypropylene or a combination thereof.

45. The apparatus of claim 35, wherein said first filter is comprised of one or more materials selected from the group consisting of hydrophobic polysulfone, hydrophilic polyether sulfone, cellulose, acetylated cellulose, nitrocellulose, polyester, polyolefin, scintered polyethylene, porous ceramics, silica, and polysaccharide.

46. The apparatus of claim 45, wherein said first filter is comprised of regenerated cellulose.

47. The apparatus of claim 46, wherein said first filter is comprised of regenerated cellulose, with an average pore size of 0.2 μm , and said frit is comprised of polyethylene or polypropylene, with an average pore size of 20 μm .

48. The apparatus of claim 35, wherein said cartridge housing preferably comprises a hollow, cylindrical or conical body, having an inlet and an outlet.

49. The apparatus of claim 48, wherein said cartridge housing is capable of being inserted into a tube, microspin tube, microfuge tube, spin cartridge, multi-well plate, vial, ampule or bag.

50. An apparatus for use in isolating biological macromolecules, comprising at least a first and second filters, wherein;

(a) said first filter comprises regenerated cellulose, with an average pore size of about $0.2\ \mu\text{m}$; and

(b) said second filter is comprised of polyethylene or polypropylene, with an average pore size of about $20\ \mu\text{m}$; and

(c) the second filter is placed in a cartridge housing; and

(d) the first filter is placed on top of the second filter; and

(e) said filters are secured to said cartridge housing with an insert.

51. A kit for use in isolating a nucleic acid molecule or a population of nucleic acid molecules, said kit comprising the apparatus of claim 32.

52. The kit of claim 51 of the invention, further comprising one or more additional compositions or reagents for use in further characterization or manipulation of the isolated biological macromolecule of the invention and a lysis composition.

53. The kit of claim 52, wherein said lysis composition is selected from the group consisting of sodium dodecylsulfate, Sarkosyl, Triton X-100, Tween 20, NP-40, N-alkylglucosides, N-alkylmaltosides, glucamides, digitonin, deoxycholate, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate (CHAPS), cetyltrimethyl-ammoniumbromide (CTAB), Brij 35, sodium iodide, sodium perchlorate, guanidine, guanidine salts, urea, lysozyme, lyticase, zymolyase, neuraminidase, Novozym 234, streptolysin, cellulysin, mutanolysin,

lysostaphin, sodium chloride, potassium chloride, magnesium chloride, lithium chloride, praseodymium, toluene, phenol, butanol, isopropyl alcohol, isoamyl alcohol, ethanol, diethyl ether, dimethyl ether, ethylmethyl ether, and chloroform, or any combination thereof.

54. The kit of claim 52 of the invention, wherein said additional compositions are selected from the group consisting of restriction enzymes, polypeptides having nucleic acid polymerase activity, cells competent for transformation, other transformation reagents, transfection reagents or other components or reagents that may be useful in conjunction with further purification, processing and analysis of the isolated macromolecules of the invention.

55. A process for isolating biological macromolecules comprising, separating a lysed natural source in a sample by filtration, wherein said sample is passed through a filter, the pore size of said filter increasing in the direction of sample flow through the filter.

56. The process according to claim 55, wherein the sample flow through the filter is promoted by applying positive or negative pressure, or by gravity, or by gravity increased by centrifugation, or by a combination thereof.

57. The process according to claim 55, wherein said nucleic acid is plasmid DNA or genomic DNA having a size of from 1 to 50 kb (kilo base pairs).

58. The process according to claim 55, wherein said sample is passed through a filter composed of a multitude of layers wherein, with respect to a particular initial pore size, the subsequent layers have increasingly larger pore sizes.

59. The process according to claim 55, wherein said sample is passed through a filter comprising at least one layer whose pore size increases in the direction of sample flow.

60. The process according to claim 55, wherein said pore size ranges from 1 μm to 500 μm , the total thickness of the filter bed being from 0.1 mm to 10 mm.

61. The process according to claim 55, wherein said sample is passed through a two-layered filter bed wherein the first filter layer has a pore size of from 0.1 to 1.0 μm , and the second filter layer has a pore size of from 1 to 500 μm .

62. The process according to claim 55, wherein said filter layers of said filter are composed of sintered polyethylene, polypropylene, polytetrafluoroethylene, glass, silica gel, alumina, or packed diatomaceous earth, e.g., cellite or silica gel, interwoven or cemented non-wovens of polypropylene, polyester, glass fibers and-silica, as well as paper, compressed paper, paper non-wovens, hydrophobic polysulfone, hydrophilic polyether sulfone, cellulose, acetylated cellulose, nitrocellulose, polyester, polyolefin, scintered polyethylene, porous ceramics, silica, and polysaccharide.

63. The process according to claim 55, wherein several samples are processed simultaneously.

64. A device for performing the process according to claim 55, comprising a cylindrical hollow body having an inlet and an outlet, disposed therein a filter comprising increasing pore sizes as seen in the direction of outlet, the filter having a pore size ranging from 0.1 μm to 500 μm , the total thickness of the filter bed being from 0.1 mm to 10 mm.

65. The device according to claim 64, wherein a hydrophobic separating layer is disposed in said cylindrical hollow body.

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